

Whole Genome Amplification: Applications Testing With the GenomePlex System



SIGMA-ALDRICH

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Abstract

The GenomePlex whole genome amplification (WGA) system offers a unique means to amplify a representative sample starting with only 10 ng of DNA. The system uses a chemical fragmentation followed by a series of stepped, isothermal primer extensions to produce a genomic library which is amplified using a limited number of PCR cycles to yield 3–5 micrograms of genomic sequence. A series of experiments have been performed to quantify representation of the WGA process demonstrating that the resulting WGA product retains the original quantitative and SNP information. GenomePlex has been successfully applied to a variety of DNA samples including damaged DNA, DNA extracted from soil, plant leaves, blood cards, and formalin fixed, paraffin-embedded tissue. Additionally, efforts to apply GenomePlex to single cell WGA suggest the method can be adapted to single cell applications.

Introduction

Genomic characterization has become central to the study of biological systems. In many instances, the genomic characterization of a sample is hampered by the availability of necessary quantities of genomic DNA. This can be particularly problematic for rare and archived samples. In an effort to immortalize the DNA from such samples, methods for amplifying whole genomes have been developed and include Linker Adaptor PCR¹, Primer Extension Pre-amplification PCR², Multiple Strand Displacement PCR³, and Degenerate Oligonucleotide Primer PCR⁴.

GenomePlex whole genome amplification is an optimized PCR variant that allows immortalization of genomic sequence from a variety of sample sources including damaged DNA, DNA extracted from soil, plant leaves, blood cards, and formalin fixed, paraffin-embedded tissue. The method is able to amplify DNA from a variety of input DNA quantities including, with optimization, single cells.

The process of GenomePlex WGA involves genomic fragmentation followed by library construction, then limited cycles of PCR. The features of the method include minimal sample pre-treatment using intact or damaged/degraded DNAs producing an unbiased genomic amplification product that can be archived and re-amplified again and again in approximately 3 hours.

GenomePlex WGA Process

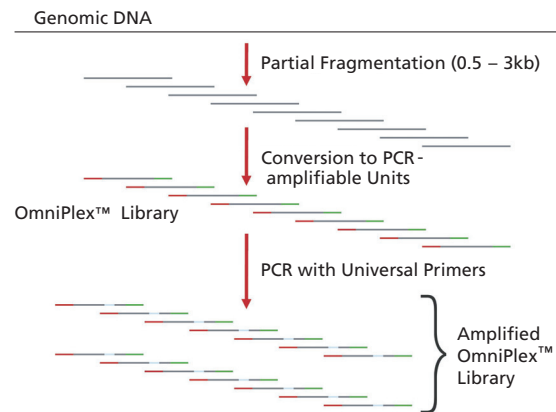


Figure 1. GenomePlex WGA steps 1) random, non-enzymatic genomic fragmentation, 2) OmniPlex library construction, converting genomic fragments into PCR-amplifiable units flanked by universal adaptor sequences, 3) limited number of PCR cycles, producing the amplified OmniPlex library suitable for subsequent use in a variety of downstream applications.

Effect of Input Template Amount

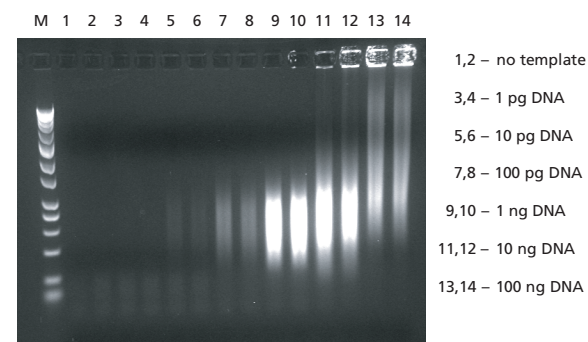


Figure 2. Gel analysis of GenomePlex WGA products from standard condition amplification reactions with increasing quantities of human genomic DNA (Roche). Marker lane (M) contains bands at 50, 100, 200, 300, 400, 500, 750, 1000, 1400, 1550, 2000, 3000, 4000, 6000, 8000, and 10000 bp. No-template controls (lanes 1, 2) and 1 pg of DNA (lanes 3, 4) yielded no products; reactions with 10 pg of input DNA (lanes 5, 6) yielded very little product (~150–750 bp) that was visible with increased UV exposure; 100 pg of input DNA (lanes 7, 8) yielded a light smear (~150–750 bp); 1 ng of input DNA (lanes 9, 10) yielded a ~150–750 bp smear; 10 ng of input DNA (lanes 11, 12) yielded a ~200–1000 bp smear; 100 ng of input DNA (lanes 13, 14) yielded a ~250 bp to >10 kb smear.

WGA Products from Various Sample Types

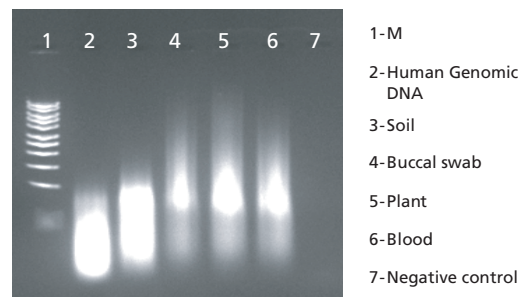


Figure 3. Gel analysis of GenomePlex WGA products from a variety of input samples. Lane 1 (M) contains bands at 10,000, 8000, 6000, 5000, 4000, 3000, 2500, 2000, 1500, 1000 and 500 bp. (Lanes 2–6) contained amplification products after DNA isolation using the GenElute™ PCR Clean-Up Kit. DNAs supplied to the GenomePlex process were isolated from the sample using standard procedures or kits. Lane 2 (positive control) was amplified from 10 ng of commercial Human Genomic DNA (Roche); lane 3 was amplification from 10 ng of DNA isolated from 500 mg of soil, lane 4 was amplified from 10 ng of DNA isolated from a Buccal swab using GenElute™ Mammalian Genomic DNA Miniprep Kit, lane 5 was amplified from 10 ng of DNA isolated from tomato leaf using GenElute™ Plant Genomic DNA Miniprep Kit, lane 6 was amplified from 10 ng of DNA isolated from fresh human blood using GenElute™ Blood Genomic DNA Kit. Lane 7 was a no DNA amplification. Higher molecular weight amplification products are generally observed for samples that have had less opportunity degradation, as in the freshly isolated samples of lanes 4–7.

Highly Representative Whole Genome Amplification with GenomePlex

A prerequisite of WGA is that a genomic sample be amplified without allelic dropout. In an effort to estimate the ability of GenomePlex WGA's ability to faithfully amplify a genomic sample, gene specific qPCR was performed using primers that sample the below human chromosomes. Each qPCR was performed on 10 ng genomic and amplified DNA. The difference between the genomic and amplified samples was expressed as the ratio of the input allelic content, calculated from the Ct's of the amplified and unamplified DNAs.

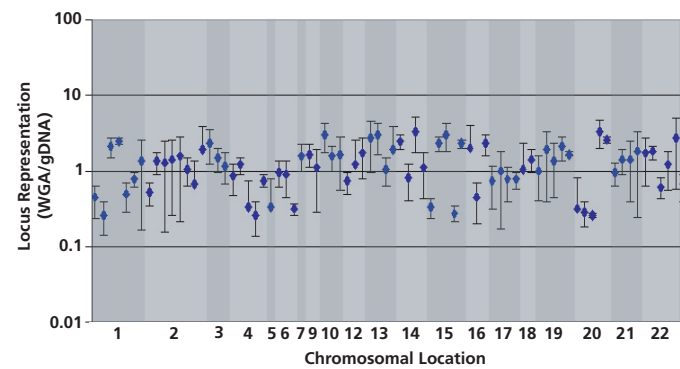


Figure 4. Graph showing allelic variance of genes from human chromosomes between GenomePlex amplified and unamplified human genomic DNA. Allelic content was calculated as 2-Ct for each qPCR product. The locus representation was calculated as WGA allelic content divided by unamplified allelic content (i.e. WGA/gDNA).

SNP Analysis and Resequencing

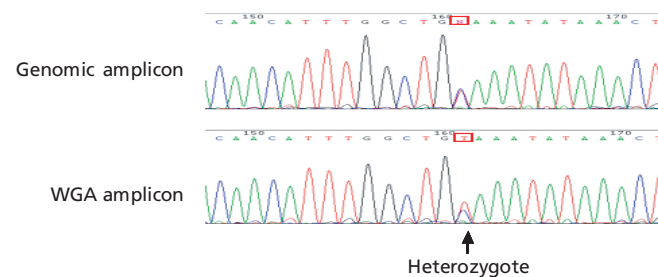


Figure 5. Sequencing electropherogram showing SNP detection of DNA isolated from human whole blood. Two samples were sequenced: a genomic amplicon (i.e., amplified directly from genomic DNA) and an amplicon generated from WGA DNA. Faithful SNP representation illustrates the usefulness of GenomePlex WGA for resequencing and related studies.

Formalin Fixed, Paraffin-Embedded Samples

Formalin Fixed, Paraffin Embedded (FFPE) samples are a difficult yet desirable sample type from which genomic information is often sought. GenomePlex WGA can be used to amplify DNA from embedded samples for genotyping and other downstream procedures.

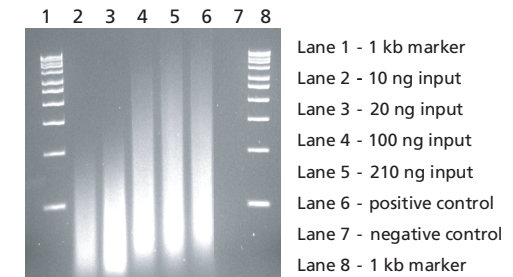


Figure 6. Amplification products from various quantities of DNA isolated from FFPE rat liver. DNA was isolated by xylene paraffin extraction followed by typical bind-and-elute DNA isolation methods. Markers in lanes 1 and 8 are as in Figure 3. Lanes 2–5 contain amplified OmniPlex library from 10, 20, 100, and 210 ng of isolated DNA respectively. Lane 6 was amplified from 10 ng of rat DNA. Lane 7 contained no added DNA during the GenomePlex process.

Allelic representation assays as described in Figure 4 performed on the 10 and 100 ng samples revealed that allelic dropout was not problematic and that representation paralleled that shown in Figure 4 for human genomic DNA.

Single Cell WGA

The lowest quantity of a complete genome is contained in a single cell. However, many current techniques are not sensitive enough to detect genomic sequence at the "single" copy level. As an example, single fibroblast cells from amniotic fluid have been gender tested using the ABI 3100 genotyping analyzer.

Optimization to attain the sensitivity required for single cell WGA included minor primer and cycling parameter modification.

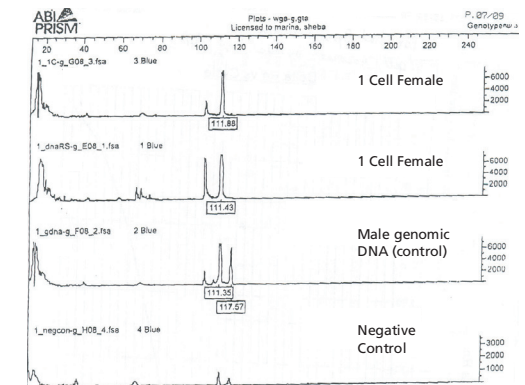


Figure 7. Gender testing results from an ABI 3100 genotyping analyzer. Single cell female results were obtained from single amniotic fluid fibroblast cells. Male genomic control DNA was unamplified DNA.

Conclusion

GenomePlex WGA is able to faithfully produce microgram quantities of genomic sequence from 10 ng (typical) of DNA, irrespective of DNA source. This was demonstrated by performing WGA on samples from a variety of sources, including those representing environmental samples (dirt), human origin (blood, buccal swab), agricultural (tomato leaves), and archived (formalin fixed, paraffin embedded rat liver). Faithful representation was illustrated by performing representation and SNP analysis on WGA vs. unamplified DNAs. These methods have been adapted to low levels of input DNA and were shown to be capable of amplifying genomic sequence from a single cell.

References

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- Zhang et al., 1992, *Proc. Natl. Acad. Sci. USA*, **89**: 5847-51.
- Dean et al., 2002, *Genome Res*, **11**: 1095-9.
- Telenius et al., 1992, *Genomics*, **13**: 718-25.

Related Sigma Products

GenomePlex® Whole Genome Amplification (WGA) Kit	Product Number WGA1
GenElute™ PCR Clean-Up Kit	Product Number NA1020
GenElute™ Mammalian Genomic DNA Miniprep Kit	Product Numbers G1N10, G1N70, G1N350
GenElute™ Plant Genomic DNA Miniprep Kit	Product Numbers G2N10, G2N70, G2N350
GenElute™ Blood Genomic DNA Kit	Product Numbers NA2000, NA2010, NA2020